

## **Application of Comprehensive Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry (GCxGC-IDTOFMS) for the Enhanced Measurement of selected POPs**

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### **Introduction**

Due to the wide spectrum of POPs, such as PCBs, PCDD/Fs, persistent pesticides, PBDEs, etc, that can be found in human matrices at significant levels, there is a need to develop high throughput specific and sensitive methods to ensure their rapid and reliable quantification. Ideally, all these compounds should be measured simultaneously in the various investigated matrices. This is, however, generally not possible due to the high number of congeners and isomers that need to be measured, as well as the large concentration range to cover. The implementation of a sample preparation step which contains all of the compounds of interest in a single extract is not attainable in practice. This is not related to any limitation of the sample preparation itself but it is actually due to the analytical tools (GC-MS) that are used at the final stage of the procedure.

To overcome the limited separation power (selectivity) inherent in the methodologies currently used to measure these compound classes, there is a need to incorporate a fractionation approach to efficiently separate analytes into sub-classes using different chemo-physical properties [1,2]. Following this fractionation process, several parallel injections have to be performed separately before the recombination of results. In order to eliminate the multiple fractionation steps, a more versatile analytical tool is needed to accommodate a multi-group analytical procedure.

Comprehensive two-dimensional GC (GCxGC) has been presented over the last 15 years as an emerging technique characterized by many advantages over single-dimensional GC [3]. Among them, the most interesting characteristics are increased peak capacity, increased sensitivity (signal enhancement following zone compression) and selectivity, independent retention processes in the two dimensions (orthogonality principle) and identification of each substance by two independent retention times [4]. GCxGC can therefore be considered as a potential tool to be integrated in a multi-group analytes procedure [5,6].

Furthermore, time-of-flight mass spectrometry (TOFMS) is suited to fast measurement of such compounds [7,8], while providing mass spectrometry detection for GCxGC. This non-mass-scanning device allows the collection of all ions at the same time and offers spectral continuity over the entire GC peak. This important feature allows mass spectral deconvolution of overlapping peaks if the fragmentation pattern is different. This technique reduces the chromatographic resolution requirements and additionally decreases the analysis time [9]. Deconvoluted ion current (DIC) can thus be used to solve chromatographic co-elution problems and TOFMS therefore acts as an analyte separation tool.

The coupling of the GCxGC chromatographic resolution capability with the deconvolution capabilities of TOFMS has been used for the measurement of a group of 58 POPs. The analyte group consists of a set of 38 PCBs, 11 persistent pesticides, 1 brominated biphenyl (BB), and 8 PBDEs that are routinely measured in human samples using various independent methodologies in our laboratory.

### Materials and methods

Experiments were carried out on the commercially available GCxGC-TOFMS Pegasus 4D<sup>®</sup> system (Leco Corp., St Joseph, MI, USA). The modulation was obtained from a 'quad jet' system (dual-stage modulator) based on the use of a dual cryogenic (liquid nitrogen cooled) cold jet and a dual hot jet installed on an Agilent 6890 GC unit.

The first dimension column was a 15m x 0.25mm i.d. x 0.25 $\mu$ m DB-1 (100% dimethylpolysiloxane) fused silica column (J&W Scientific, Folsom, CA, USA) and the second dimension column was a 1.5m x 0.10mm i.d. x 0.1 $\mu$ m HT-8 (8% phenyl (equiv.) polycarborane-siloxane) fused silica column (SGE Int., Ringwood, Australia). The two GC columns (dimensions) were connected together via a glass press-fit connector (Restek, Bellefonte, PA, USA). The modulation was performed on the second dimension column itself at an offset temperature of 60°C regarding the primary GC oven. The primary GC oven was programmed as follows: 90°C for 1 min, then to 150°C at 10°C/min, then to 250°C at 3°C/min, then to 290°C at 5°C/min and held for 2 min. The second dimension column was coiled in the secondary oven that was 50°C higher than the primary oven (iso-ramping mode). The modulator period was 4 seconds (0.25Hz modulation frequency) and the hot pulse duration was 600 ms. Helium was used as carrier gas at a constant flow of 1 ml/min.

The transfer line connecting the secondary column and the MS source was operated at a temperature of 250°C. The source temperature was 250°C with a filament bias voltage of -70V. The data acquisition rate was 60 scans per second for a mass range of 100 to 750 amu. The detector voltage was 1800V. Data processing and display of the GCxGC chromatograms were achieved using the Leco ChromaTOF<sup>™</sup> software.

Injection volumes were 1 $\mu$ L splitless. The standard containing the 58 native analytes and 42 <sup>13</sup>C-labeled homologues was made from EC-502, ES-5019-CS1-8 and EO-5159 solutions obtained from CIL (Andover, MS, USA).

### Results and Discussion

The GCxGC contour plot chromatogram of the 58 selected analytes is illustrated in Figure 1. One can see that GCxGC solved the single-dimensional co-elutions of PCB-118 and PCB-149, PCB-105 and PCB-153, PCB-128 and PCB-183, BDE-47 and PCB-172, PCB-170 and Mirex, BB-153 and BDE-154. Over the entire set of compounds, PCB-74/Heptachlor epoxide and PCB-196/PCB-203 were not chromatographically resolved. However, due to the analytical resolution of the TOFMS, the mass deconvolution of PCB-74 and Heptachlor epoxide was achieved and permitted separate peak identification. PCB-196 and PCB-203 were not deconvoluted since these analytes are characterized by the same fragmentation pattern. Therefore, 56 analytes were separately identified of the 58.

The column set was selected after testing various phase combinations. The DB-1/HT-8 column set had the advantage that it could be used at fairly high temperature, which, in addition to relatively short column lengths, was useful for the elution of high molecular mass compounds. Half height peak width in the second dimension was 80-150ms.

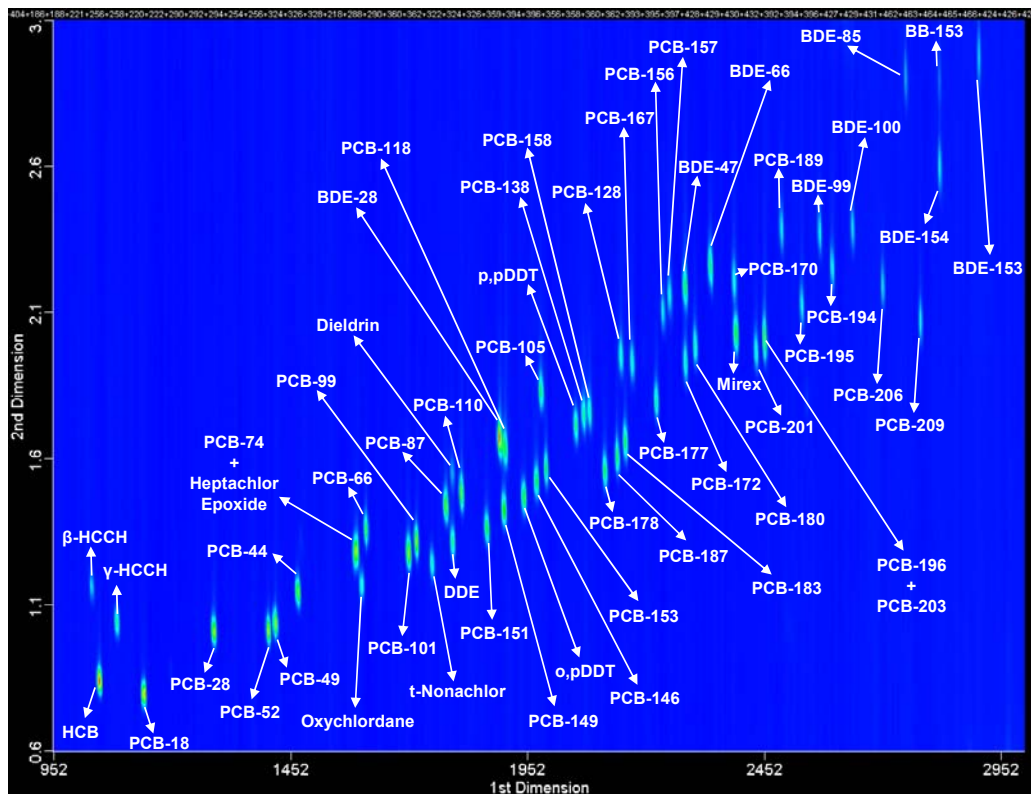


Fig. 1: GCxGC/TOFMS chromatogram (contour plot) of the 58 compounds based on analytes characteristic ions.

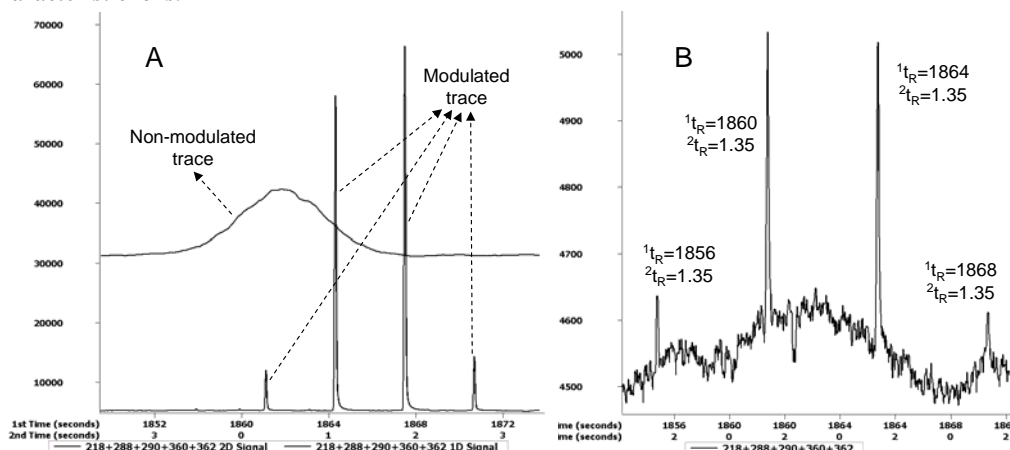


Fig. 2: Both Figures are based on the reconstructed trace of PCB-151, A) Signal enhancement following modulation (zone compression), B) chromatographic 'slices' for a 5pg/ $\mu$ l injection ( $^1t_R$  and  $^2t_R$  are retention times in first and second dimensions in seconds).

Considering the quantitative aspect, Figure 2A illustrates the net signal enhancement effect achieved using GCxGC with TOFMS as the detector. A 9-fold enhancement in signal amplitude can be observed, resulting from a compromise between signal enhancement following zone compression due to modulation and loss in sensitivity due to the high data acquisition rate of the TOFMS. Figure 2B shows the signal recorded for a 5pg/ $\mu$ l splitless injection. The 4 slices can easily be integrated, and the sum of the values represents the signal used for quantification. The instrument LODs range between 0.5pg and 5pg through the mass range of compounds.

Calibration curves can be semi-automatically constructed for each of the 58 analytes. Fairly good coefficient values are obtained (Fig. 3) for concentrations ranging from 1pg to 1ng.

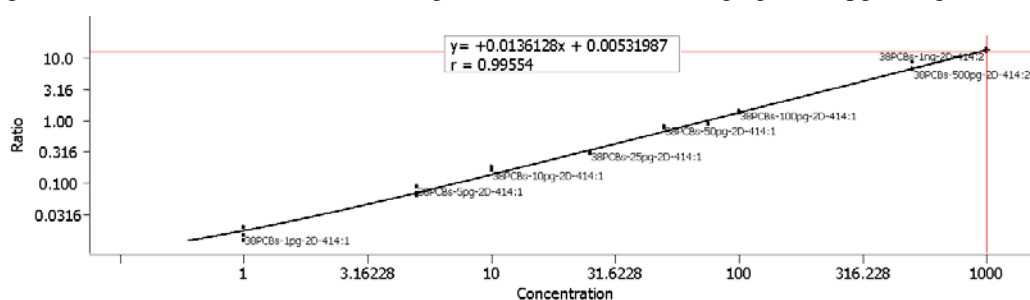


Fig. 3: GCxGC/IDTOFMS calibration curve for PCB-153 based on 5 characteristic ions for native and labeled analytes.

### Conclusion

The separation of 38 PCB congeners, 11 persistent halogenated pesticides, 1 BB, and 8 PBDEs has been achieved using comprehensive multidimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-TOFMS). The chromatographic resolution of GCxGC coupled with the analytical resolution of the TOFMS produces a powerful combination that solves most of the potential co-elution problems.

### Acknowledgements

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